

Different Learning Stabilization Processes between Wakefulness and Sleep

 Aaron Cochrane, Kiley Haberkorn, Takeo Watanabe, and  Yuka Sasaki

Department of Cognitive & Psychological Sciences, Brown University, Providence 02912

Visual perceptual learning (VPL) is initially fragile and must be stabilized after training to enable long-term retention. While sleep-dependent stabilization has been linked to changes in excitation/inhibition (E/I) balance, the neural mechanisms supporting stabilization during wakefulness remain unclear. Here, we investigated whether electrophysiological indices associated with sleep-based stabilization also characterize stabilization during posttraining wakefulness. Humans of both sexes completed visual training tasks with two competing training blocks separated by an awake rest period during which electroencephalography (EEG) was recorded. We conducted two studies targeting different stabilization states: one in which learning was vulnerable to retrograde interference and a control condition in which learning was largely stabilized through extended training and rest. Across studies, we estimated theta-band power and a criticality-based EEG index of E/I balance from posterior cortical sites. When stabilization was incomplete, elevated E/I balance during wakeful rest was associated with greater subsequent interference, whereas reduced E/I balance predicted stronger stabilization, paralleling findings previously reported during REM sleep. In contrast, when learning was fully stabilized, elevated E/I balance was associated with continued performance improvement rather than interference. Theta power showed a distinct, state-dependent pattern, predicting interference during wakefulness but not after stabilization. These findings indicate that wakeful stabilization of VPL shares a common inhibitory-dominant mechanism with REM sleep, while theta power is related to stabilization in different directions depending on whether the brain is wake or in REM sleep. Together, the results suggest that stabilization during wakefulness and REM sleep share partially common but not completely the same processing.

Key words: criticality; detrended fluctuation analysis; EEG; perceptual learning; resting state; retrograde interference

Significance Statement

To allow for healthy functioning, human perception must display plasticity in response to some experiences (i.e., beneficial learning) and stability in the face of other experiences (i.e., resilience to maladaptive learning). Our visual systems face this challenge of balancing plasticity and stability throughout every day of our lives, yet the regulation of plasticity during wakefulness is poorly understood. Here we use recently developed electrophysiological approaches to scalp EEG, in conjunction with a behavioral retrograde interference paradigm, to compare awake plasticity and stability to the excitation/inhibition balance changes in sleep that characterize plasticity and stability. Our results show that wakefulness and sleep share stabilization characteristics under certain circumstances but not others.

Introduction

Humans greatly rely on vision in most aspects of life, and our ability to see is built up through many experiences in the world in which we tune our perceptual faculties (Herzog and Efeld, 2009). Such

plasticity in visual processing, or visual perceptual learning (VPL), which is defined as long-term performance improvement due to visual experience (Seitz and Dinse, 2007; Watanabe and Sasaki, 2015), is a key component in allowing the rich diversity of human perceptual expertise. It may be surprising, then, how easily VPL can be interfered with or disrupted, thereby preventing lasting benefits of learning. After an initial period of learning there is a critical posttraining period during which learning must be stabilized. This stabilization, defined as resilience to retrograde interference, involves posttraining neural processes and occurs not only in VPL but also in almost all forms of learning (Dudai, 2004; Robertson et al., 2004; Seitz et al., 2005; Shibata et al., 2017). While stabilization plays an important role in long-term retention, the mechanisms underlying this process remain poorly understood.

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Correspondence should be addressed to Aaron Cochrane at aaron_cochrane@brown.edu.

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Behavioral studies using two competing training blocks suggest that the stabilization process during posttraining periods takes multiple forms across wakefulness and sleep. During wakefulness, immediately after VPL training, early visual areas remain highly plastic and are therefore susceptible to retrograde interference from a competing second training block (Seitz et al., 2005; Shibata et al., 2017; Bang et al., 2018). However, the learning acquired in the first training block becomes resilient to such retrograde interference within a few hours, allowing learning of the second training to occur (Shibata et al., 2017). Interestingly, during posttraining sleep, non-rapid eye movement (NREM) sleep increases the plasticity of presleep VPL while being largely independent of interference, whereas rapid eye movement (REM) sleep stabilizes presleep VPL (Tamaki et al., 2020).

Given that real-world learning is often followed by an extended period of wakefulness, and that stabilization may follow different courses across wakefulness and sleep, it is crucial to determine whether stabilization in these states relies on the same neural mechanisms. A previous study demonstrated that during posttraining REM sleep, stronger lateralized theta power (5–7 Hz) in the posterior cortical regions near early visual areas was associated with greater stabilization of VPL trained before sleep (Tamaki et al., 2020). That study also used magnetic resonance spectroscopy (MRS) to quantify excitatory and inhibitory metabolites in early visual areas and computed their ratio as an index of E/I balance. They found that decreased E/I balance during REM sleep relative to wakefulness was linked to greater stabilization of presleep VPL. Thus, these findings suggest that REM sleep stabilization of VPL is associated with both increased theta power and decreased E/I balance (Fig. 1).

The present study investigated whether the stabilization process during wakefulness relies on mechanisms similar to those engaged during REM sleep, with a particular focus on electrophysiological signatures. Recent work has suggested that electroencephalographic (EEG) measures can serve as estimates of E/I balance (Bruining et al., 2020; Ahmad et al., 2022; Diachenko et al., 2024). Supporting this idea, we recently found that EEG-derived estimates of E/I balance were positively correlated with MRS-derived E/I balance during sleep (Cochrane et al., 2025). Since the electrophysiological neural bases of VPL stabilization during wakefulness remain poorly understood, we tested whether theta power and theta-based indices of E/I balance were associated with the stabilization process of VPL during posttraining wakefulness.

Materials and Methods

Participants. All procedures complied with the protocol #1203000582 approved by the Brown University Institutional Review Board. Screening criteria were applied to ensure that participants were neurologically and psychologically healthy, between the ages of 18 and 60, and with normal or corrected-to-normal vision. The numbers of participants in each study were determined based on the sequential Bayes factors framework (Schönbrodt et al., 2017) that allowed flexible sampling plans with fixed cutoff criteria described below. To estimate a Bayes factor, we used a Bayesian rank correlation between lateralized theta power and interference, which had been found in sleeping humans post-learning (Tamaki et al., 2020). After an initial small sample of participants was collected, further data collection proceeded until the Bayes factor (Wagenmakers, 2007; Morey et al., 2015) in favor of either the null or the correlation reached 3:1 (i.e., a $BF_{\log}$ less than -1 or greater than 1). We recruited 20 subjects for Study 1 and 30 subjects for Study 2 and report the behavioral results of these participants. Several participants' EEG-based E/I balance could not be estimated due to limitations of the estimation algorithm (Bruining et al., 2020; Diachenko et al., 2024) and were thus excluded from bivariate

analyses (see below). The resulting sample with both EEG and behavioral data had a total of 45 subjects (16 for Study 1 and 29 for Study 2), and a mean age of 26.9 years ($SD = 12.0$), with 38 females.

Experimental methods. We conducted two studies in order to target two periods of learning stabilization. Study 1 aimed to measure EEG-based E/I balance during the interval that would be followed by retrograde interference, whereas Study 2 aimed to measure EEG-based E/I balance during the interval that would be followed by minimal interference. While our primary interest was in the mechanisms of stabilization (Study 1), we considered a comparison to fully stabilized learning to provide a valuable control condition (Study 2).

The present studies were preregistered, with the primary results reported here corresponding to those described in the preregistration (AsPredicted #136395; aspredicted.org/3bt8-zqrd.pdf). The preregistration describes our experimental methods, sampling procedure, analytical methods, and anticipated results. However, our primary methods differ from the preregistration in two notable ways. First, we completed two separate studies with similar methods following the methods and analytical plans of the preregistration, whereas in the preregistration, only one study was described. Second, in the preregistration we erroneously listed sigma band as our primary interest. This was incorrect because (1) the manuscript referred to in the preregistration found that increased resilience to interference was related to theta activity during REM sleep, not sigma (Tamaki et al., 2020), and (2) the sigma band is not a canonical EEG frequency band in awake humans; it is primarily of interest during sleep (Uchida et al., 1991). A third minor change was also implemented: while the preregistration lists 3 min EEG recording windows, we instead utilized 3.5 min EEG recording windows.

We used a texture discrimination task (TDT) that is common in the field of VPL (Karni and Sagi, 1991; Censor and Sagi, 2008; Tamaki et al., 2020). In this task, participants identified the horizontal or vertical configuration of a set of three diagonally oriented target lines in the visual periphery while maintaining fixation at the center of the display (Fig. 2B). At the center of the display, there was a fixation task ("is the character an L or T?"), and at all other locations in a 19×19 grid, there were either vertically or horizontally oriented task-irrelevant lines. This display remained on the screen for 33 ms. At a certain latency, the stimulus-to-mask onset asynchrony (SOA), a mask array appeared after the target array. The location of the target array was the same for all blocks of a given participant and pseudorandomly counterbalanced across participants.

Each study involved two perceptual training blocks on this discrimination task with informative auditory feedback on each trial (Training A and Training B; Fig. 2A). Training A and Training B used orthogonal background orientations (horizontal or vertical, pseudorandomly counterbalanced across participants), which has been associated with gains on Training A being interfered with by Training B, if they were performed in close succession (Yotsumoto et al., 2009). However, if there is an interval intervening between two training blocks, stabilization of learning of Training A may occur. The two training blocks were each preceded and followed by test blocks in which the same background orientation as Training A was used without feedback (see below). The two training blocks were separated by an awake rest period during which EEG was recorded across four equally spaced periods of 3.5 min of passive fixation (see below).

Study 1 included 300 trials of training during each of two training blocks (Fig. 2A), whereas Study 2 included 375 trials of training. In both studies, a series of SOAs was adaptively determined for training, instead of fixed SOA values commonly used for all participants, as we wished to match the task difficulty across participants. During the initial 100 trials of every block, each SOA was initially chosen on each trial to be randomly either 50, 150, or 300 ms. Then, a Weibull psychometric function was fit using maximum-likelihood optimization on the preceding 100 trials' data to determine the empirical three SOA values at which a percent correct of 60, 75, or 90% was expected. From 101st trial onward, on each trial one of these three empirically defined SOA values was chosen randomly for training. Participants' head positions were stabilized using a chin rest at 60 cm from the monitor.

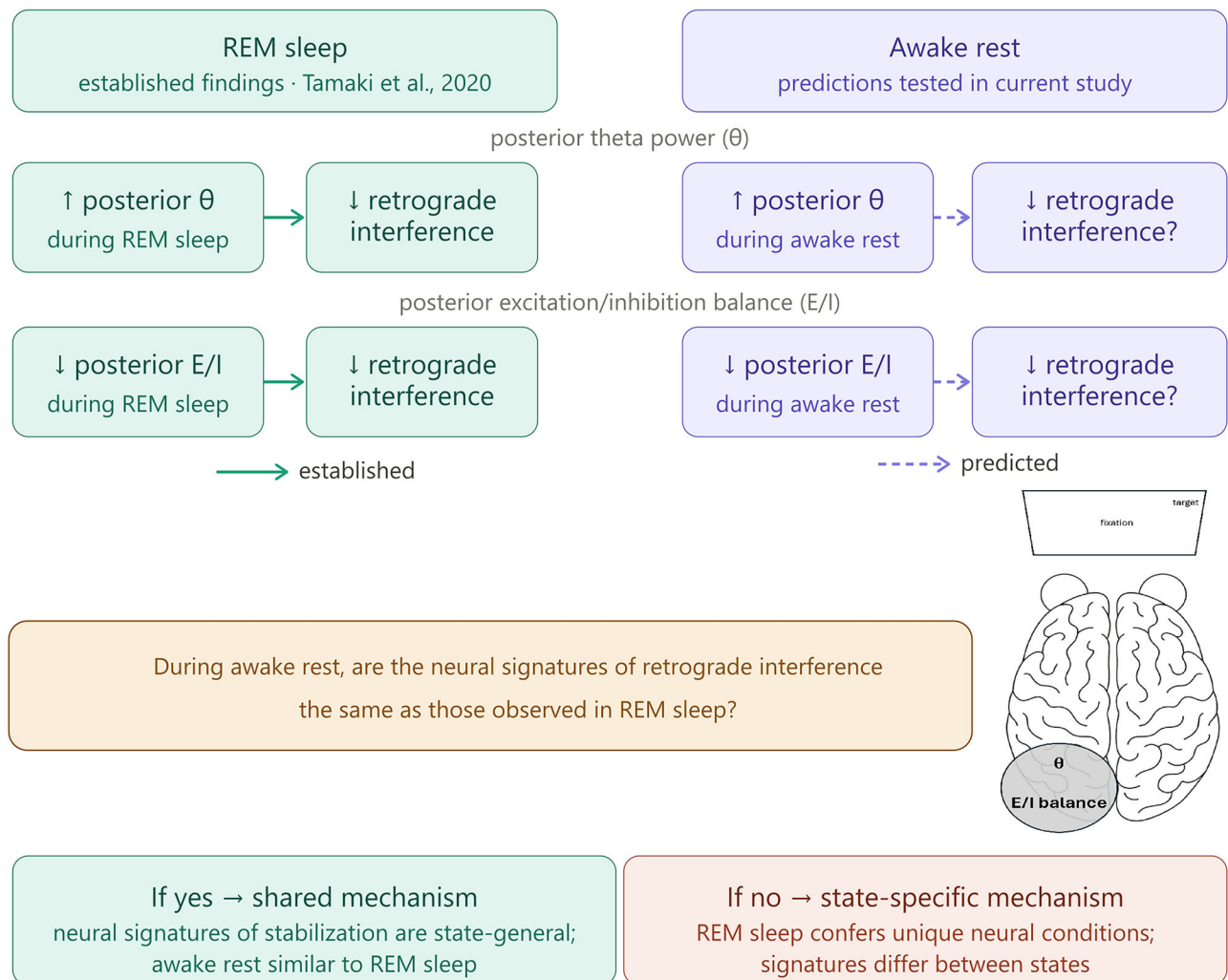


Figure 1. Study schematic. Given previously observed results linking brain states in REM sleep to a reduction in retrograde interference, we test the proposition that shared mechanisms of learning stabilization occurs in awake rest.

Four test blocks of 90 trials each were used to maintain maximally similar methods to Tamaki et al. (2020). Performance was represented by the threshold SOA at which 75% accuracy of the orientation discrimination was made (see threshold estimation below). In each trial, one of three SOAs (60%, 75%, and 90%-SOA, defined above) was randomly chosen. The change of threshold SOA from Test 3 to Test 4 was of primary interest (see preregistration aspredicted.org/3bt8-zqrd.pdf). This is because, after completing Training A and then having EEG measured during a subsequent awake resting period, completing Training B could interfere with the task performance of Training A. The primary behavioral measure of interest was interference, which would be shown as worse performance of Training A (longer threshold SOA) after completing Training B, or stabilization, which would be shown as a lack of diminished performance of Training A after completing Training B. The performance change was a single dimension that was measured as the proportional change in threshold from Test 3 to Test 4 (i.e., Test 4 threshold minus Test 3 threshold, divided by Test 3 threshold). We report our results such that positive values indicate increases in threshold (i.e., interference) while negative values indicate decreases in threshold (i.e., stabilization).

Threshold estimation. Behavioral performance was quantified as the threshold (in seconds SOA) required to achieve 75% accuracy in the orientation discrimination task during test blocks. This preregistered choice of threshold was due to 75% being halfway between guessing rate and ceiling performance, thereby being an interpretable value to represent

the full sigmoid Weibull function. Instead of using conventional maximum-likelihood estimation of the psychometric function for each participant separately, we employed a Bayesian hierarchical estimation of the psychometric function that pooled all participants' data. This approach provides a principled, robust, and flexible framework to characterize performance in psychophysical tasks (Cochrane et al., 2024; Zhao et al., 2024). This is because the hierarchical Bayesian framework incorporates the overall tendency across all participants when estimating individual thresholds, leading to more stable and precise estimates than separate maximum-likelihood fits for each participant.

We implemented this procedure using a Bayesian generalized nonlinear mixed-effects model in the R package *brms* (Bürkner, 2017), which hierarchically estimated thresholds at each of the following levels: single trials, 30-trial mini-blocks, and participants. In the *brms* model, first, accuracy was modeled as a function of SOA with a nonlinear Weibull psychometric function incorporating a fixed lapse rate of 0.005 (Wichmann and Hill, 2001; Cochrane and Green, 2023). Second, the Weibull shape parameter was estimated with by-participant random intercepts, whereas the threshold parameter was estimated with a fixed effect of task phase, a random intercept for each combination of participant and 30-trial mini-block (Zhao et al., 2024), and a random intercept for each trial independent of participants [in Wilkinson notation: $\text{threshold} \sim \text{taskPhase} + (1 \mid \text{subjectMiniblockID}/\text{subjectID}) + (1 \mid \text{totalTrialNum})$]. This model structure allowed the estimation of a unique threshold for every single trial, with participant-level thresholds varying across mini-blocks. Finally, fitted trial-level thresholds were extracted from the Bayesian model and averaged

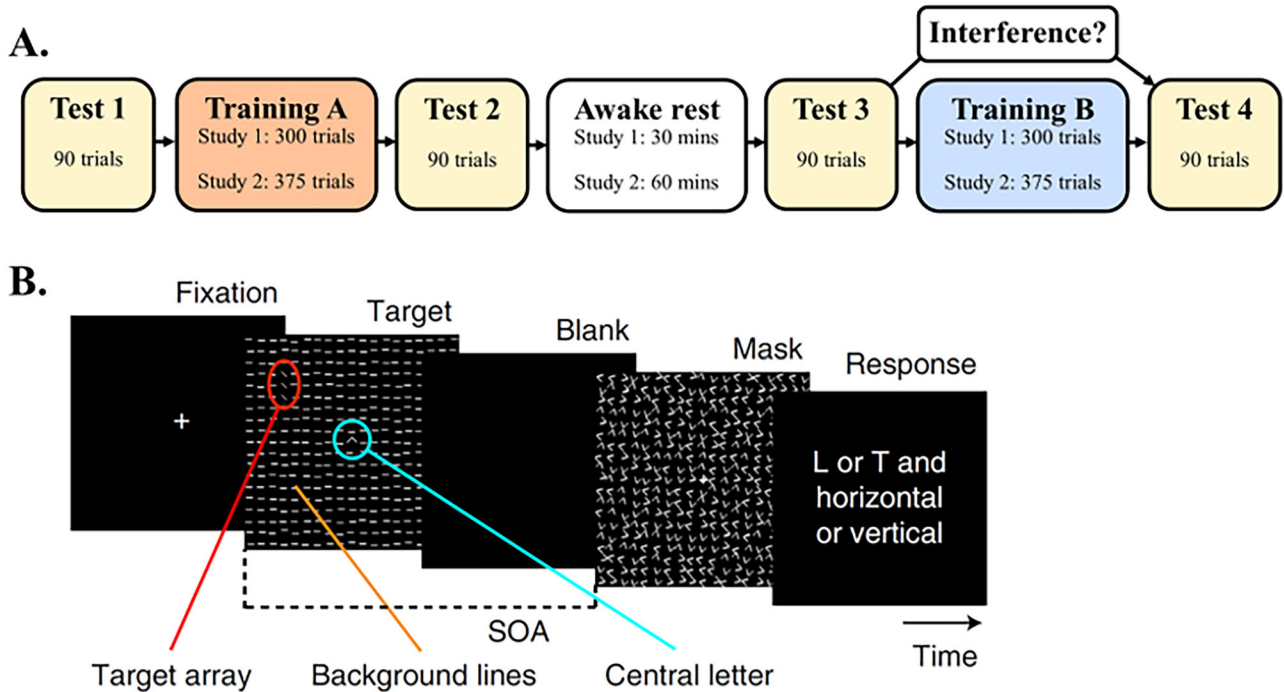


Figure 2. Experimental design. **A**, Participants completed two perceptual training blocks (Training A and Training B); one training block used vertical background lines and the other horizontal background lines. The primary behavioral variable of interest was the proportional change in 75% threshold SOA from Test 3 to Test 4, which would indicate either stabilization or interference due to completing Training B. The awake rest included four equally spaced 3.5 min periods of passive fixation. **B**, Texture discrimination task design. A fixation task, identifying a central letter as a rotated L or T, was completed at the same time as texture discrimination task, identifying the vertical or horizontal configuration of a set of diagonal oddball lines (targets) within the display. SOA modulates task difficulty. Panel **B** was adapted from Tamaki et al. (2020).

as needed for the reported results (e.g., to obtain participant-level means for each test block). Only trials in which the fixation task was answered correctly were analyzed.

EEG. We used a 20-channel active-electrode EEG montage (Brain Products ActiCap Slim) sampling at 1,000 Hz. All EEG analyses followed the previous study by Tamaki et al. (2020) in reporting the differences between the mean of the three most posterior contralateral-to-trained channels and their corresponding ipsilateral-to-trained (lateralized) channels (channels analyzed were O1, PO3, PO7, O2, PO4, and PO8) for each measure. As in the previous study (Tamaki et al., 2020), these posterior channels were interpreted as neural activity lateralized to the processing of the target stimuli, thereby providing baseline correction. During passive fixation periods resting-state EEG was recorded. Total resting duration was 30 min in Study 1 and 60 min in Study 2.

EEG data from the rest period with passive fixation windows was first preprocessed using EEGLAB (Delorme and Makeig, 2004) by high-pass filtering at 0.1 Hz, low-pass filtering at 50 Hz, rereferencing to global mean of the 20 channels, downsampling to 250 Hz, automated artifact rejection using the *clean_rawdata* EEGLAB plugin (which includes automated window and channel rejection algorithms), and then Infomax independent components analysis (ICA; Makeig et al., 1997; Delorme et al., 2012). Infomax ICA is an analytical technique used to separate mixed EEG signals into a set of independent components, each representing a putatively unique source (either neural activity or a specific type of artifact, such as eye or muscle movements). Each ICA component was subsequently manually inspected by evaluating its topographical distribution on the scalp and its frequency characteristics including power spectra. Components were rejected if their features were consistent with non-neural sources, including strong power in the low-frequency range localized frontal electrodes for eye-related artifacts, or strong power in the high-frequency range for muscle activity.

Theta power. First, we identified the channels contralateral and ipsilateral to the trained visual field for each participant.

Second, in a processing step shared with the E/I balance estimation (see below and Bruining et al., 2020), we obtained theta power for each of the six posterior EEG channels at each of four timepoints using a finite impulse response filter via the *firfilt* EEGLAB plugin, from which the amplitude envelope was computed using a Hilbert transform. Third, we averaged the theta power at each of contralateral and ipsilateral channels and then obtained the lateralized theta power (contralateral minus ipsilateral). Finally, we averaged lateralized theta power across all timepoints for each participant.

EEG-E/I balance. We estimated excitation/inhibition (E/I) balance based on EEG measures, as we have previously shown that the EEG-based estimates of E/I balance using the following algorithm are reliably and positively associated with E/I balance estimated using MRS, which measures excitatory and inhibitory metabolite concentrations (Cochrane et al., 2025). We employed a method derived from criticality theory, which considers biological systems to operate at the cusp of state transitions, wherein these systems can efficiently transition into subcritical (stable and inhibition-dominant) or supercritical (chaotic and excitation-dominant) regimes (Hardstone et al., 2012; Poil et al., 2012; Shew and Plenz, 2013; Geertjens et al., 2022). This method, which we refer to as EEG-E/I balance or simply E/I balance, decomposes the timecourse of a frequency band's (theta activity in the present paper) detrended amplitude envelope into components of self-affinity and amplitude and uses the relations between these two measures to identify the EEG signal's generation by subcritical or supercritical (i.e., inhibition-dominant or excitation-dominant, respectively) neural activity (Bruining et al., 2020; Yang et al., 2023; Kat et al., 2024). More precisely, the processing steps for this method involve (1) bandpass filtering, (2) Hilbert transform (amplitude envelope estimation), (3) mean-centering, (4) calculating the cumulative sum, (5) separating into sliding windows of a predetermined length (see below), (6) normalizing these windows by their corresponding original amplitudes, (7) detrending each window, (8) estimating the standard deviation in each window, (9) calculating the product-moment correlation between each

window's standard deviation and its original amplitude, and (9) subtracting the resulting correlation coefficient from 1. This provides a measure of E/I balance which varies from 0 to 2, with higher values indicating greater excitation-dominance. Put differently, the method considers recording periods (e.g., 3 min) and windows of time within that recording period. If, within the recording period, windowed signal amplitude and detrended variability are negatively correlated, then the E/I regime being recorded is excitation-dominant. If signal amplitude and detrended variability are positively correlated, then the E/I regime being recorded is inhibition-dominant (see Bruining et al., 2020 for full explanation of algorithm, simulations, and pharmacological validation). Estimation of detrended fluctuation analysis (DFA) exponent occurs as well, after bandpass filtering, in order to determine that the signal properties are appropriate for the analyses or whether recording periods should be excluded (Diachenko et al., 2024). Similar methods have been used to identify altered E/I balance in patient populations such as autism spectrum disorder (Juarez Martinez et al., 2019) and Alzheimer's disease (van Nifterick et al., 2023). Accordingly, we used this EEG-based E/I metric as a functional index of excitation-inhibition balance, supported by both empirical validation with MRS and its theoretical grounding in criticality theory.

We estimated E/I balance from the amplitude envelope of the canonical theta frequency band and used window sizes of 15 s with an overlap of 0.8. See Diachenko et al. (2024) for further information on the robustness of the algorithm to variations in these hyperparameters. DFA exponent, a related self-affinity measure linked to the scale-free temporal dynamics of biological signals, was also applied to the EEG data (Bruining et al., 2020; Diachenko et al., 2024). As recommended by the originators of this EEG-E/I algorithm, we therefore excluded E/I estimates arising from EEG windows with a DFA <0.6. This process of excluding windows of EEG data in response to DFA exponents being <0.6 led to all windows of EEG data being excluded for some participants; as a result, our final sample of EEG data included 16 participants (Study 1) and 29 participants (Study 2) for whom we averaged their nonexcluded E/I balance estimates. For secondary analyses, because DFA exponents (sometimes called Hurst Exponents; Stanyard et al., 2024) are theoretically linked to E/I balance, although not in a monotonic manner (Bruining et al., 2020), lateralized DFA exponent values were computed using the same averaging and subtraction method. For a visualization of patterns of E/I balance change over the rest period, see Figure S1.

We obtained the lateralized E/I balance during the awake interval in a similar way as the lateralized theta power. Specifically, we calculated the E/I balance for each of the six EEG channels given the amplitude envelopes described above. Then, we averaged the E/I balance for the contralateral and ipsilateral sides to the trained visual field, respectively, and subtracted the ipsilateral value from the contralateral value. Finally, we averaged E/I balance across all timepoints for each participant.

Statistical methods. All correlations we report here were based on Spearman rank correlations (ρ), providing robustness to outliers. To

assess evidence for or against the presence of a correlation we used $\text{BF}_{\log_3}$, the base-3 log of the Bayes factor; in this formulation, a value of less than -1 corresponds to a conventional “at least substantial evidence for a null effect,” whereas a value of >1 corresponds to “at least substantial evidence for a non-null effect” (Wagenmakers, 2007). Values between -1 and $+1$ are equivocal (i.e., not able to be easily interpreted). Bayes factors were estimated using the `correlationBF` function from the `BayesFactor` package in R (Morey et al., 2015) with all priors being specified as “wide” within that function. We also report the bootstrapped 95% confidence intervals of each correlation. Further, we treat each ρ over $|0.3|$ as being of worth noting to potentially guide future investigation (Funder and Ozer, 2019), regardless of the corresponding Bayes factor.

Results

Due to the similar behavioral results observed in prior work on VPL stabilization and sleep (Seitz et al., 2005; Yotsumoto et al., 2009; Tamaki et al., 2020), we predicted that stabilization of VPL during wakefulness would reflect the same underlying mechanisms as those occurring during REM sleep (Tamaki et al., 2020). These mechanisms would be reflected by two patterns of results (Fig. 1). If theta power is involved in stabilization, then the more theta power, the more resilience to interference (more improvement). If E/I balance is involved in stabilization, then the lower E/I balance, the more resilience to interference (more improvement).

Study 1

Behavioral stabilization results

We first confirmed that participants learned TDT using a pair-samples t test comparing Test 1 (mean threshold = 0.15) to Test 2 (mean threshold 0.08; $T = -6.11$, $p < 0.001$). By comparing participants' average thresholds during Test 4 to Test 3, we observed that participants ($n = 20$) demonstrated a large variation in stabilization, with over half of participants (12/20) showing interference (Fig. 3). When examining effects of the rest period itself (i.e., changes from Test 2 to Test 3), we also observed that 12 participants' thresholds increased. Further, when comparing thresholds in Test 4 relative to Test 2, we observed that 11 participants' thresholds increased, indicating that there was no net learning observed after Test 2.

Correlations between threshold changes and overall EEG measures during the rest hour

In Study 1 we observed strong positive correlations between interference (threshold increase) and lateralized theta power (i.e., contralateral minus ipsilateral; $n = 16$, $\rho = 0.606$; $\text{CI}_{95} =$

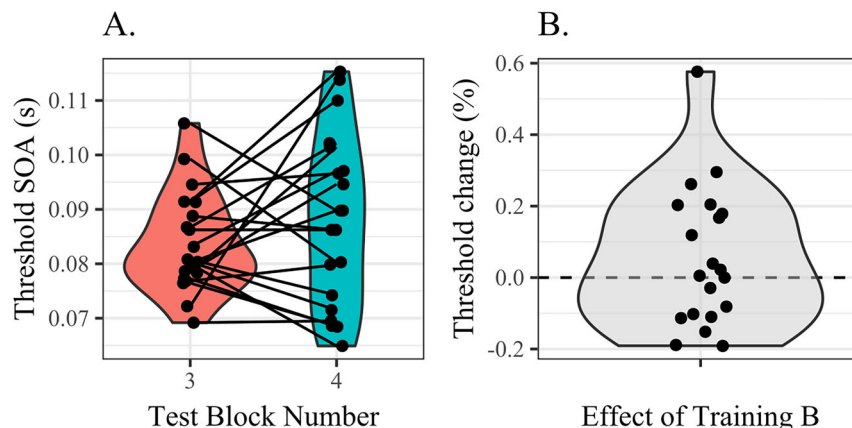


Figure 3. In Study 1, thresholds (in seconds of SOA) necessary to achieve 75% correct in test block 3 and test block 4 (A), and the differences between these two values (normalized as a proportion of threshold in test block 3; B). Lower values indicate superior performance (A) and superior stabilization of learning (B). Each point represents a single participant.

[0.07,0.92]; $BF_{103} = 1.6$; Fig. 4A; this surpassed our sampling stoppage criterion). This was not consistent with a previous study (Tamaki et al., 2020), in which they found that interference was associated with less theta power, thus the opposite relationship. However, we found a similar link between stabilization and lowered E/I balance ($n = 16$, $\rho = 0.571$; $CI_{95} = [0.03, 0.86]$; $BF_{103} = 1.2$; Fig. 4B) in the awake stabilization to the link during REM sleep found by Tamaki et al. (2020). Further, unlike the links between REM theta power, E/I balance, and interference, here we found that lateralized theta power was not strongly associated with E/I balance ($\rho = 0.171$; $CI_{95} = [-0.35, 0.60]$). Because the DFA exponent reflects the temporal structure of the theta-band amplitude envelope, we examined whether the relationship between threshold change and EEG-based E/I balance could be accounted for by the DFA exponent alone. We found no substantial evidence for a correlation between threshold change and the DFA exponent ($\rho = 0.206$; $CI_{95} = [-0.34, 0.67]$). Thus, performance change was strongly associated with the EEG-based E/I balance, but not with the DFA exponent alone. We next examined whether the correlation between E/I balance and threshold change was restricted to our lateralized measure or was present when instead examining the geometric mean between E/I balance estimates from ipsilateral and contralateral channels; in this case there was no reliable association between the behavioral and neural measures ($\rho = -0.429$; $CI_{95} = [-0.81, 0.16]$).

These results showed that the modulation of E/I balance associated with stabilization was common during wakefulness and REM sleep, whereas the theta power alone showed the opposite direction during wakefulness and REM sleep.

Study 2

Methodological changes

Study 1 provided evidence that learning stabilization occurs during wakefulness via similar changes in E/I balance as occur during REM sleep. However, there remained the possibility that the interindividual variability in performance change in Study 1 was not reflecting stabilization per se; instead, it could be due to variability caused by test–retest effects or vigilance changes during long recordings. Therefore, we conducted a control experiment wherein we ensured that learning was fully stabilized. We thus used the same preregistered methods and sampling procedure as Study 1 but with longer training and rest periods in Study 2.

Behavioral stabilization results

Study 2, when compared with Study 1, had longer training (375 rather than 300 trials) and longer rest (60 rather than 30 min) in

order to induce full stabilization but was otherwise identical. As with Study 1, participants improved from Test 1 (mean threshold = 0.16) to Test 2 (mean threshold = 0.08; $T = -10.68$, $p < 0.001$). In Study 2, very few participants demonstrated interference (2/30) after the 60 min interval. That is, most participants improved from Test 3 to Test 4 (Fig. 5A), suggesting learning of Training A was mostly stabilized and resilient to interference. When assessing proportional change from Test 2 to Test 3, we found that 24 participants' thresholds increased over the rest period. Further, when comparing thresholds in Test 4 relative to Test 2, we observed that 16 participants' thresholds increased, indicating that there was no net offline learning observed after Test 2.

Correlations between threshold changes and overall EEG measures during the rest hour

There was evidence against a correlation between lateralized theta power and interference ($n = 29$, $\rho = 0.028$, $CI_{95} = [-0.36, 0.4]$, $BF_{103} = -1.1$; Fig. 6A; this satisfied our preregistered sampling stoppage criterion). In contrast, there was strong evidence for an association between lateralized E/I balance and reduced interference ($n = 29$, $\rho = -0.519$, $CI_{95} = [-0.77, -0.15]$, $BF_{103} = 2.4$; Fig. 6B), with elevated lateralized E/I balance being linked to increased improvements in performance from Test 3 to Test 4. That is, we found that increased E/I balance was associated with continued task improvement despite the presence of a potentially interfering task. When testing whether the relationship between threshold change and EEG-based E/I balance could be accounted for by the lateralized DFA exponent alone, there was no evidence for a correlation between threshold change and DFA exponent ($\rho = -0.012$; $CI_{95} = [-0.4, 0.4]$). Similarly, there was no evidence for a correlation with nonlateralized E/I balance and interference (i.e., geometric mean of ipsilateral and contralateral E/I balance estimates; $\rho = -0.20$; $CI_{95} = [-0.61, 0.29]$). Thus, performance change was strongly associated with the lateralized EEG-based E/I balance, but not with the DFA exponent alone.

These results suggest that the significant association between performance changes and E/I balance in Study 1 cannot be explained by a mere test–retest effect or the passage of time. If the association had simply reflected test–retest variability or fatigue, we would have expected similar correlations in both Studies 1 and 2. Instead, manipulating the degree of stabilization by increasing the number of trials and the interval altered the slope of the relationship between the performance change and E/I balance, indicating that the association depends on the stabilization process itself.

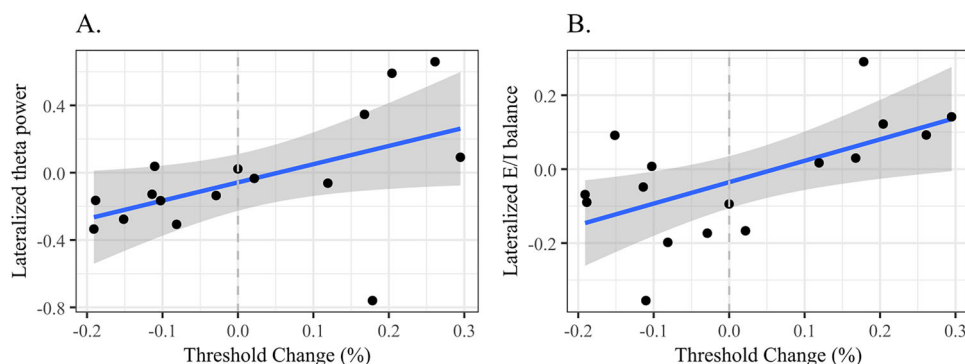


Figure 4. Correlations between proportional change in threshold from Test 3 to Test 4, on the x-axes, and lateralized EEG measures, on the y-axes. **A**, Theta power; **B**, EEG-based excitation/inhibition balance. Each point represents a single participant, averaged across all EEG measurement windows.

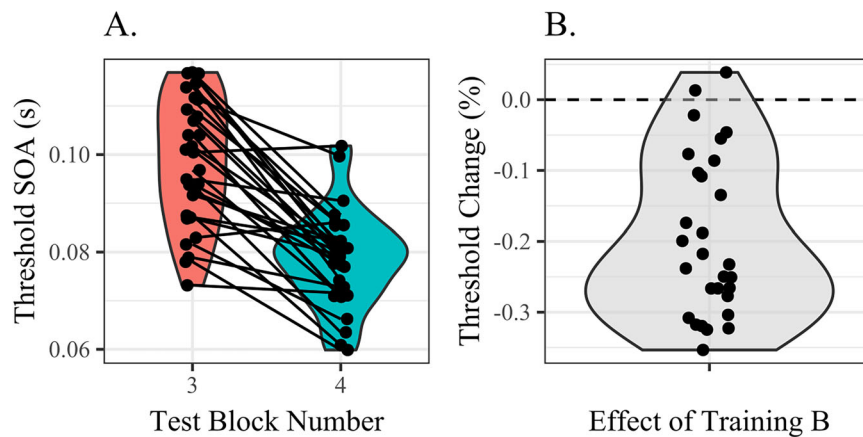


Figure 5. In Study 2, thresholds (in seconds of SOA) necessary to achieve 75% correct in test block 3 and test block 4 (**A**), and the differences between these two values (normalized as a proportion of threshold in test block 3; **B**). Lower values indicate superior performance. Each point represents a single participant.

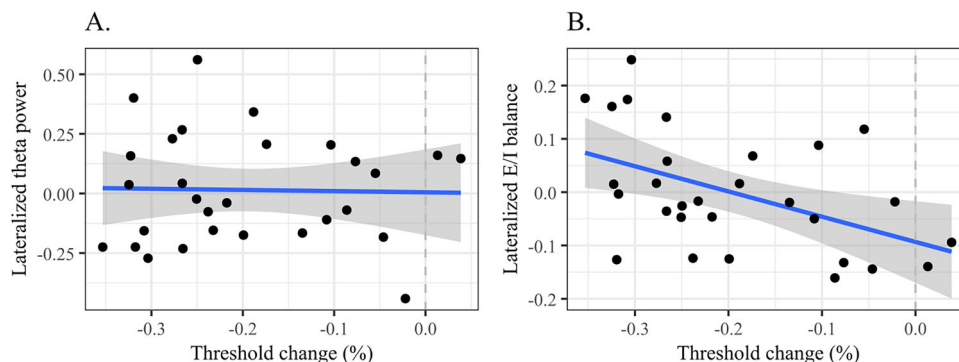


Figure 6. Correlations between EEG measures during rest and normalized change in threshold. **A**, Theta power; **B**, EEG-based excitation/inhibition balance. Each point represents a single participant, averaged across all available EEG measurements.

Discussion

Investigating the mechanisms that stabilize VPL is essential for understanding how neuroplasticity yields lasting functional changes. However, in contrast to stabilization during sleep, relatively little is known about how the brain stabilizes VPL during wakeful rest. One possibility is that similar mechanisms underlying stabilization operate during wakefulness and REM sleep. To examine this possibility, we induced neuroplasticity through VPL training, recorded EEG during a brief posttraining rest period, and then measured interindividual differences in susceptibility to interference. By linking electrophysiological indices including theta-band amplitude and theta-based estimates of E/I balance to these behavioral differences, we directly compared the mechanisms underlying stabilization during wakefulness with those previously observed during REM sleep. Our findings indicate that awake stabilization and REM sleep stabilization share some, but not all, underlying neural mechanisms (Fig. 7).

E/I balance-related findings

Study 1, in which interference was introduced before stabilization had fully occurred, showed that awake stabilization involves E/I balance changes similar to those reported during REM sleep (Tamaki et al., 2020). Because stabilization unfolds over time, we conducted Study 2 as a control, in which stabilization was expected to be largely completed. In this condition, the REM sleep-like E/I balance change observed in Study 1 did not occur. Importantly,

both studies involved multiple test sessions, yet sleep-like E/I balance effects were observed only in the condition where active stabilization was still taking place. This indicates that the modulation of E/I balance in Study 1 cannot be attributed to mere test–retest effects but rather reflects electrophysiological signatures of the stabilization process itself. Furthermore, lower E/I balance was associated with stronger stabilization, consistent with findings from REM sleep, suggesting that reduced E/I balance constitutes a shared mechanism supporting the stabilization of learning.

Theta power-related findings

In contrast to REM sleep, where stronger stabilization was linked to stronger theta power (Tamaki et al., 2020), we observed the opposite pattern during wakefulness. In Study 1, greater theta power during posttraining rest predicted greater interference, not greater stabilization. In Study 2, when learning was fully stabilized, the correlation between theta power and performance change was close to zero. These findings indicate that theta power plays fundamentally different roles in stabilization during wakefulness and REM sleep. Theta activity has been linked to top-down or feedback communication within cortical hierarchies (Hyafil et al., 2015; Mahjoory et al., 2020). In animal memory research, theta dynamics have also been proposed to differentiate information-processing modes across wakefulness, NREM sleep, and REM sleep (Hasselmo, 1999, 2005). Thus, the opposite directions of the stabilization–theta relationships observed across

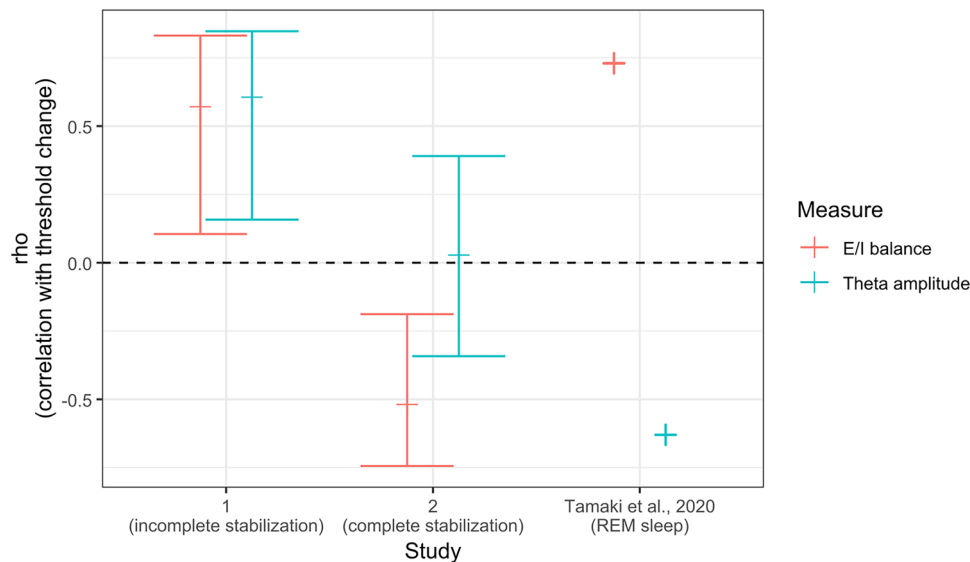


Figure 7. Summary of results. Here we summarize the results of Study 1 and Study 2. Plotted are the rank correlations between threshold change (Test 3 to Test 4) and each of the two EEG measures. Error bars indicate 95% CI of rho. We additionally plot the corresponding point estimates of correlations reported in Tamaki et al. (2020; REM sleep; note that E/I balance was measured using MRS).

wakefulness and REM sleep may reflect state-dependent differences in feedback signaling that support stabilization. Such a distinction may itself be indicative of fundamental differences between sleep and wakefulness that underpin previously observed differential consequences for learning outcomes (Karni and Sagi, 1993; Mednick et al., 2003; Censor et al., 2006).

One may wonder whether Study 2 reflects an overlearning-like state, given its extended training and rest periods. We argue that this is unlikely, because E/I balance did not decrease after the first training block in Study 2 (Fig. S1), whereas overlearning in Shibata et al. (2017) was characterized by decreases in E/I balance following training. In addition, Shibata et al. (2017) reported that reductions in E/I balance, mediated by increased inhibitory metabolite levels, were accompanied by strong anterograde interference, referred to as hyperstabilization. In contrast, in Study 2, E/I balance was not correlated with performance during the second training block, which would be indicative of anterograde interference ($\rho = 0.189$; $CI_{95} = [-0.21, 0.54]$). These findings suggest that the effects of E/I balance observed in Study 2 reflect a physiological process distinct from the GABA-mediated hyperstabilization associated with overlearning reported in the previous study (Shibata et al., 2017) and are therefore unlikely to be attributable to over-practice per se. Last, we observed a lack of offline improvements in Study 2 (i.e., most participants' thresholds increased from before the rest period to after the rest period), which indicates a lack of hyperstabilized learning immediately following the training period.

Our results of Study 2 may reflect a case of facilitation rather than interference. That is, although VPL typically does not lead to transfer from one training task to another, sequential training has been shown to produce facilitatory transfer-like effects under certain conditions (Xiao et al., 2008; Kattner et al., 2017; Bruns et al., 2025). While our dataset is not well positioned to be definitive on this topic, we speculate that our E/I balance results may provide one mechanistic explanation for such sequential facilitation effects. Our results indicate that, after an initial period of training and stabilization, a shift into a more excitatory-dominant regime may allow the perceptual system to utilize new training on a second task to continue to tune the

improvements observed on the first task. The two tasks we used had shared attributes, such as temporal structure and spatial allocation of attention, and we propose that a poststabilization increase in E/I balance may have allowed overlapping task demands to be trained and thus lead to facilitatory effects on the initially trained task (Cochrane and Green, 2023). Further research would be needed to directly test this proposition.

The current results also support the broader use of EEG-based estimates of E/I balance for addressing key questions in neuroplasticity (see also Diachenko et al., 2024; Kat et al., 2024; Avramiea et al., 2025; Cochrane et al., 2025). By replicating the link between reduced E/I balance and reduced plasticity previously identified in sleep using MRS (Tamaki et al., 2020), the present findings highlight the utility of criticality-based EEG signatures for indexing cortical processes involved in neuroplasticity (Bruining et al., 2020).

Despite our ability to effectively address our core question regarding the mechanisms of stabilization in wakefulness, our results carry several key limitations. First, although our single-day methods are standard for testing VPL stabilization, they remain limited to a relatively short training period. VPL is often studied over the course of multiple days of training, and future methodological innovations would be required to study stabilization phenomena on these longer timescales. Second, all of our participants were healthy adults, which provides a useful sample for establishing VPL phenomena, but may not be highly generalizable to important patient or aging populations. Third, we did not measure potentially confounding factors such as drowsiness, which could have influenced both neural and behavioral measures. Finally, while we would have liked to test each recorded EEG window separately, the variability in the EEG measurements from 3.5 min windows would mean that we would have required a much larger sample size to conduct such analyses (Fig. S1). Future research would benefit from refining methods for higher-powered EEG analyses to allow for tests of dynamic changes within the rest period.

We have shown that visual plasticity, which is initially fragile after training, can be stabilized within ~ 1 h through reductions in E/I balance during wakeful rest. Although this finding aligns

with stabilization processes identified during REM sleep, open questions remain regarding how awake- and REM-related stabilization interact with stabilization arising from extensive training (Shibata et al., 2017) and with processes occurring during NREM sleep (Tamaki et al., 2020).

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